

Food plant toxicants and safety; Risk assessment and regulation of inherent toxicants in plant foods.

Citation for published version (APA):

Essers, A. J. A., Alink, G. M., Speijers, G. J. A., Alexander, J., Bouwmeister, P., van den Brandt, P. A., Ciere, S., Gry, J., Herman, J., Kuiper, H. A., Mortby, E., Renwick, A. G., Shrimpton, D. H., Vainio, H., Vittozzi, L., & Koeman, J. H. (1998). Food plant toxicants and safety; Risk assessment and regulation of inherent toxicants in plant foods. *Environmental Toxicology and Pharmacology*, 5(3), 155-172.
[https://doi.org/10.1016/S1382-6689\(98\)00003-9](https://doi.org/10.1016/S1382-6689(98)00003-9)

Document status and date:

Published: 01/01/1998

DOI:

[10.1016/S1382-6689\(98\)00003-9](https://doi.org/10.1016/S1382-6689(98)00003-9)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Download date: 05 May. 2023

Assessment

Food plant toxicants and safety

Risk assessment and regulation of inherent toxicants in plant foods¹

A.J. Alexander Essers ^a, Gerrit M. Alink ^a, Gerrit J.A. Speijers ^b, Jan Alexander ^c,
Pieter-Jan Bouwmeister ^d, Piet A. van den Brandt ^e, Sandra Ciere ^f, Jørn Gry ^g, John Herrman ^h,
Harry A. Kuiper ⁱ, Eileen Mortby ^j, Andrew G. Renwick ^k, Derek H. Shrimpton ^l,
Harri Vainio ^m, Luciano Vittozzi ⁿ, Jan H. Koeman ^a

^a Department of Food Technology and Nutritional Sciences, Agricultural University, Wageningen, Netherlands

^b Centre for Substances and Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

^c Department of Environmental Medicine, National Institute of Public Health, Oslo, Norway

^d Commission of the European Communities, DG XII Agro Industrial Research, Brussels, Belgium

^e Department of Epidemiology, University of Maastricht, Maastricht, Netherlands

^f Department of the Environment, Quality and Health, Ministry of Agriculture, Nature Management and Fisheries, The Hague, Netherlands

^g Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration, Søborg, Denmark

^h International Programme on Chemical Safety, World Health Organisation (WHO), Geneva, Switzerland

ⁱ State Institute for Quality Control of Agricultural Product (RIKILT-DLO), Wageningen, Netherlands

^j Food Contaminants Division, Ministry of Agriculture, Fishery and Food (MAFF), London, UK

^k Clinical Pharmacology Group, University of Southampton, Southampton, UK

^l Consultant in Nutrition and Food Science, Cambridge, UK

^m Unit of Chemoprevention, International Agency for Research on Cancer, Lyon, France

ⁿ Department of Comparative Toxicology and Ecotoxicology, Rome, Italy

Accepted 28 January 1998

Abstract

The ADI as a tool for risk management and regulation of food additives and pesticide residues is not readily applicable to inherent food plant toxicants: The margin between actual intake and potentially toxic levels is often small; application of the default uncertainty factors used to derive ADI values, particularly when extrapolating from animal data, would prohibit the utilisation of the food, which may have an overall beneficial health effect. Levels of inherent toxicants are difficult to control; their complete removal is not always wanted, due to their function for the plant or for human health. The health impact of the inherent toxicant is often modified by factors in the food, e.g. the bioavailability from the matrix and interaction with other inherent constituents. Risk-benefit analysis should be made for different consumption scenarios, without the use of uncertainty factors. Crucial in this approach is analysis of the toxicity of the whole foodstuff. The relationship between the whole foodstuff and the pure toxicant is expressed in the 'product correction factor' (PCF). Investigations in humans are essential so that biomarkers of exposure and for effect can be used to analyse the difference between animals and humans and between the food and the pure toxicant. A grid of the variables characterising toxicity is proposed, showing their inter-relationships. A flow diagram for risk estimate is provided, using both toxicological and epidemiological studies. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Risk assessment; Inherent food plant toxicants; Natural toxins; Whole foodstuff toxicity

¹ Correspondence to: Nicole G. Ch. Wevers, ETAF, European Training and Assessment Foundation, PO Box 182, 6700 AD Wageningen, The Netherlands. Tel.: +31 317 484924; fax: +31 317 484941.

1. Introduction

Traditional foods are supposed to be safe on the basis of long term experience, even though these foods may contain inherent toxicants and anti-nutritional substances. For considering the safety of food derived from biotechnology, the OECD (1993) described its concept of food safety based on long-term experience: 'The safety of food for human consumption is based on the concept that there should be a reasonable certainty that no harm will result from intended uses under the anticipated uses of consumption. [...] In principle, food is presumed to be safe unless a significant hazard has been identified.' The OECD concept relates to the past understanding of risk and health demands. However, contemporary advances in analytical techniques and our growing knowledge of long-term effects and effects from chronic exposure make it possible to detect health effects which were previously unrecognised. Furthermore, our standard of living, life expectancy and health perception have changed considerably over the past few decades. This includes cultural changes in diets of consumers, with trends such as organic, vegetarian, exotic, minimally processed and fast foods. This all necessitates reviewing the previously considered safety of products against the present situation.

The desire to breed for plant resistance, combined with new biological techniques that may produce drastic alterations of plant metabolite levels, as well as new food processing methods, have resulted in the development of new plant food commodities. In the evaluation procedures for novel food plants and products thereof, the demonstration of substantial equivalence to an existing plant or product is a corner stone. In this context, inherent food plant toxicants are important substances to be selected for this comparison of equivalence. These new opportunities for both the enhancement as well as the reduction of plant metabolites also raise questions as to the desirability or necessity to alter them. For some substances it may therefore be necessary to evaluate the possible risks and, possibly, to set limits.

An important question is whether food plant toxicants should be treated differently from man-made chemicals in risk assessment and regulation. In 1995 the US Committee on Comparative Toxicity of Naturally Occurring Carcinogens (National Research Council, 1996) concluded, after assessing the relative potency of over 200 carcinogens of which 65 occurred naturally, that there is no notable mechanistic or potency difference between synthetic and the known naturally occurring carcinogens in the human diet. The Committee then concluded that both naturally occurring and synthetic chemicals can be evaluated by the same epidemiology or experimental methods and procedures.

However, the above mentioned approach is inappropriate in most cases for inherent food plant toxicants,

as it does not take into consideration the accompanying toxicity-modulating food factors such as possible health beneficial constituents, matrix effects, or the overall health benefit of the food.

The main objective of this paper is to discuss the appropriateness of applying the current methods in risk assessment as a basis for risk management and regulation in this context, and a more appropriate approach for inherent plant toxicants is proposed.

Inherent food plant toxicants are plant constituents which might give rise to adverse effects in humans when the plant or plant products are ingested. This definition was originally adopted by EU-AIR-NETTOX in 1996 (Gry et al., 1998) and it is comparable with the definition by the USA Food and Drug Administration: a naturally occurring poisonous or deleterious substance is an inherent natural constituent of a food which is not the result of environmental, agricultural, industrial, or other contamination (Ely, 1989).

Anti-nutritional factors are food components that diminish or inhibit the adequate utilisation of specific nutrients. In general these constituents are not toxic themselves, but limit the bioavailability of nutrients. These anti-nutritional factors are not taken into consideration in the present document.

1.1. Role of inherent toxicants in the plant

Inherent plant toxicants are among the plant metabolites which are claimed to have an ecological role in the physiology, proliferation or defense of plants. Although some constituents seem to be designed to deter feeding by mammals, which are thus toxic or otherwise unpleasant to humans, several constituents with possibly other purposes (plant physiological, defense against insects, bacteria, fungi and viruses) may also just happen to be toxic to humans (Harborne, 1988). Of most substances, however, their precise function for plant health and proliferation is not known. This makes modifying their levels in plants in the pursuit of minimising risks of food poisoning a delicate matter.

Some of these constituents are allelopathics or phytoalexins. Induction of many of such inherent toxicants can result from a plant's exposure to many kinds of elicitors, e.g. bacterial infection, viruses, exposure to cell wall fragments, cold, UV light, heavy metal salts, antibiotics, fungicides, herbicides and at feeding sites of nematodes (Beier, 1990).

1.2. Health protecting and adverse effects

Several plant constituents may have certain positive health effects under some conditions, and adverse effects under others. An example of this is the apparent oestrogen agonistic or antagonistic effect of plant oestrogens such as isoflavones in soybean. Neonatal

soy-genistein administration prevented mammary cancer in rats (Lamartiniere et al., 1995). Associations have been found between soybean consumption and reduced breast cancer in women, and consequently, claims of breast cancer prevention have been made (Adlercreutz and Mazur, 1997). On the other hand, neonatal exposure to phytoestrogen diets altered the uterus weight in female rats, as well as the neuroendocrine development in both male and female rats (Whitten et al., 1993, 1994; Medlock et al., 1995; Whitten et al., 1995). The type of effects may depend upon the phytoestrogen type, dosage, timing, duration and pattern of exposure and the age of the consumer. The high exposure of infants to phytoestrogens from soy-based infant formula is likely to exert biological effects (Setchell et al., 1997).

Another example is the flavonoid quercetin which, in experimental studies has been shown to have carcinogenic effects (Zhu and Liehr, 1994) and to be an anti-carcinogen (Deschner et al., 1991; Scambia et al., 1991), while epidemiological studies indicate protection against cardiovascular diseases (Hertog, 1994). Certain glucosinolates may affect thyroid, liver, and kidney function, while some metabolites of glucosinolates have been shown to activate mechanisms which moderate or reverse carcinogenic processes (Heaney and Fenwick, 1995).

After reviewing the literature on cruciferous plants, Beier (1990) concludes that the glucosinolate degradation product indole-3-carbinol (I3C) isolated from *Brassica oleracea* given *before* a carcinogen, protects against carcinogenesis, whereas, when given *after* a carcinogen, it strongly promotes carcinogenesis. Substances which alter the microsomal mixed-function oxidase enzyme system may increase the detoxification of some carcinogens but enhance the neoplastic response under other conditions (Wattenberg, 1979; De Flora et al., 1991).

Of a number of naturally occurring organic substances their capacity to prevent the neoplastic effects of chemical carcinogens has been demonstrated in experimental studies (Wattenberg, 1979; De Flora et al., 1991). Current activities are especially focused on the following groups of constituents: glucosinolate degradation products, flavonoids, carotenoids, simple coumarins, terpenoids, cystein sulphoxide degradation products, curcuminoids and cinnamic acid derivatives. These compounds may also pose a toxic potential. It is likely also that other inhibitors are present in natural products.

Saponins are usually classified as inherent food plant toxicants. Recently it was found that soybean saponins in mice prevented the induction of preneoplastic changes in the colon (Korotkar and Rao, 1997). The authors suggested that soybean saponins might play a significant role as chemotherapeutic substances in the management of colon carcinogenesis.

Reduction of the anti-nutritional factor phytic acid in the human diet has been advocated in order to counteract the problems of mineral availability (Liener, 1980), while others refer to the potential role of phytate in reducing colon cancer and plead for it to remain (Messina, 1992).

Apart from the essential nutrients, there is no hard proof (yet) that other food constituents have a beneficial net effect, but their possible positive effect on health should not be ignored.

In cases where the necessity of a constituent has not been proven and as long as the substance has been shown to produce adverse effects in well performed experiments, it seems prudent to consider the adverse, rather than the possible health promoting effects, for risk assessment. However, the margin between the levels which result in positive or negative health effects, respectively, as well as intake in the population, should be established before intake levels are regulated. Last but not least, care should be taken with the extrapolation from high-dose experiments in animals to real intake levels in humans, while the net effect of the food product on the uptake and metabolism of the compound, as discussed below, is critical in the evaluation.

2. The basis for risk management and regulation of food additives and contaminants

2.1. Types of toxicity and risk assessment

Toxic effects may be divided into two broad types, deterministic and stochastic. *Deterministic* effects typically increase in severity with increase in dose, and so show a dose-dependent frequency distribution in the exposed population; typically, a threshold dose exists below which an effect is not produced. Most toxic effects are deterministic. *Stochastic* effects show an increase in incidence with increase in dose, but the severity of the effect is largely independent of dose. Stochastic effects, such as the effect of a genotoxic carcinogen, are considered not to show a threshold dose. Therefore, the higher the dose, the more likely it is that an individual will show the adverse effect (IPCS, 1994).

Traditionally, different approaches have been adopted in order to ensure human safety. For deterministic effects, the NOEL (no-observed effect level) has been used as a surrogate for the threshold for toxicity with uncertainty factors used to determine an acceptable daily intake (ADI) or tolerable daily intake (TDI) (see below). For stochastic effects risk assessment has either used a mathematical model, such as linear, multi-stage model, to predict the dose associated with a specified low risk (e.g. 1 in 10⁶ lifetimes) or the absence of a threshold has been used as a basis for recommen-

duction of a reduction in exposure to the lowest level technologically possible. Dose-response extrapolation outside the range of the experimental data is critically dependent on the mathematical model used; uncertainty increases and precision decreases, the further the risk estimate is from the experimental range.

2.2. The ADI concept, history and definition

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met for the first time in 1956 to consider the safety of food additives. During the first few meetings the concept of the acceptable daily intake (ADI) was developed, which was adopted by the Committee in 1961. The ADI is derived from a no-observed-effect-level (NOEL) to which a safety factor is applied (FAO/WHO, 1958). In scaling from experimental animals to humans, JECFA has based the relationship on body weight, and the ADI is expressed in terms of mg/kg body weight per day.

JECFA continues to establish ADIs for food additives and veterinary drugs. Likewise, the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) establishes ADIs for pesticides. JECFA uses the same approach for contaminants that exhibit a threshold of toxicity, although other terminology that employs the concept 'tolerable' rather than 'acceptable' is used.

The ADI has been defined (FAO/WHO, 1991) as "*an estimate of the amount of a substance in food or drinking water, expressed on a body-weight basis, that can be ingested daily over a lifetime without appreciable health risk (standard human = 60 kg).*"

Regulations or international standards for chemicals in food should be established in such a way that the ADI is not exceeded when the regulation or standard is met. Both JECFA and JMPR express the ADI in a range, from zero to its numerical value. The purpose is to emphasise that no more of a food additive should be used than necessary to achieve its desirable technological effect (Good Manufacturing Practice) or of a pesticide or veterinary drug than is necessary under Good Agricultural Practice or Good Practice in the Use of Veterinary Drugs, respectively. This is known as the ALARA principle, *as low as reasonably achievable*.

The ADI is based on the weight of evidence approach in that all available information is considered in its establishment. The NOEL that is used as the basis for the ADI is usually the highest one for the critical effect in the most sensitive species (IPCS, 1994). When evaluating toxicological data it is not always obvious whether the effects that are observed are adverse. This is particularly true with food additives where reductions in body weight are often observed. JECFA usually takes the conservative approach that an effect should be considered adverse in the absence of information to the contrary and should serve as the basis for the ADI.

With pesticides, the effects observed are nearly always adverse, so the term 'no-observed-adverse-effect-level (NOAEL)' is used.

The NOAEL is not a precise indication of the threshold level dose at which toxic effects occur in the test animals. The NOAEL depends on the background incidence of the effect, the sensitivity of the measurement, the spacing of the doses and the size of the group. An alternative starting point for risk assessment is the benchmark dose (BMD), which is the 95th percent lower confidence limit of the dose producing a 1, 5 or 10% response determined by dose-response modelling (Barnes et al., 1995). The BMD makes greater use of the dose-response data but, like the NOAEL, it does not address the uncertainties of inter-species differences or inter-individual variability in the human population, nor the extrapolation from high to low dose.

The ADI is based on data from toxicity/safety studies in animals, or humans when available. The NOAEL derived from the studies is divided by a safety or uncertainty factor to allow for *any species differences in susceptibility, the numerical differences between the test animals and the human population exposed to the hazard, the greater variety of complicated disease processes in the human population, the difficulty of estimating the human intake and the possibility of synergistic actions among food additives* (IPCS, 1987). In practice a factor of 100 is usually applied to the NOAEL from animal studies and ten to the data from human studies. In consequence the factor of 100 is regarded as comprising two factors of ten; one ten-fold factor to allow for inter-species differences and the other ten-fold factor to allow, primarily, for human variability. These factors are applied to the external dose (mg/kg bw/day) and allow for uncertainties in both kinetics (delivery to the target site) and dynamics (sensitivity of the target site to the chemical delivered via the circulation for systemic toxicity). Thus the interspecies factor is to allow for undefined, possible differences between the test animal and humans and the inter-individual factor is to allow for undefined possible variability between humans (Renwick, 1991).

Reviewing the adequacy of the 10×10 safety factor, Renwick (1991) concluded that the 100-fold safety factor is difficult to justify on theoretical grounds, but it remains a pragmatic solution to the interpretation of animal data. This seems to be a generally accepted view among toxicologists.

It has been proposed that each ten-fold uncertainty should be further subdivided into toxicokinetic and toxicodynamic aspects, so that chemical specific data could be incorporated into the risk assessment in order to replace a default uncertainty factor by relevant data (Renwick, 1993). This concept was discussed at a WHO Task Group meeting (IPCS, 1994), which concluded that the ten-fold factor for species differences could be

considered to comprise four-fold for kinetic aspects and 2.5-fold for dynamics, and that the ten-fold factor for interindividual differences could be considered as comprise two equal factors (3.16×3.16). This reconsideration of the role of the 100-fold uncertainty factor provides a mechanism for the incorporation of the results of recent progress in toxicology, such as toxicokinetics, mechanisms of toxicity, Pharmacologically Based Pharmacokinetic (PB-PK) modelling, in vitro data and biomarkers of exposure or effect. Elucidation of the kinetics and mechanisms of toxicity will help to improve the extrapolation of results from high dose experiments in rodents or in vitro systems to estimates of risk associated with low dose exposure in the human situation. This helps in species selection and the development of biomarkers. Similar knowledge is also important in the risk assessment of food plant toxicants as described in detail below.

The concept of the ADI is a generally accepted and widely adopted system to protect the population from potential adverse effects of food additives and pesticide residues. However, the use of a large factor to allow for the uncertainties of inter-species and inter-individual differences may not be appropriate to considerations of an inherent toxicant present in a plant food.

3. Safety evaluation and regulation of inherent food plant toxicants—problems with the application of the ADI concept for evaluating inherent food plant toxicants

ADI's have not been established for most inherent food plant toxicants, because no NOAEL values have yet been established in animal models, because of the vast number of substances in plants and the lack of economic stimulus to do so. A complicating factor is that some inherent food plant toxicants, in addition to their adverse effects, may exercise health protecting effects, depending on the dosage, the target organ and the consumer's physical condition and age. Also, the presence of other factors, contained in the same food, may greatly influence the possible effects. The ADI is established at a value at which effects are very unlikely to occur in humans. The default options (i.e. usual assumptions made in the interpretation of toxicological data) are generally viewed as providing a 'conservative estimate' of risk; that is, their use is likely to overestimate the true risk. Because the ADI is applied to substances that are added to food, safety can be ensured by limiting levels of use or by prohibiting those substances.

The margin of safety between the intake of inherent food plant toxicants and the appearance of toxic effects is often quite low. Their removal is not always desirable, due to their possible function in the plant's physi-

ology, proliferation or defence, and as they are sometimes linked to desired organoleptic traits of the food. Also for some substances, such as micro nutrients, an intake slightly below a toxic one may be beneficial for some health aspects. The use of standard uncertainty factors would limit the intake of the plant material to such a low level that the use of the food would, in practice, be prohibited. Thus, risk/benefit decisions must be made.

The risk manager ultimately must decide, for example, which population groups are most 'at risk', whether the food supply is sufficient to limit a particular product, and which percent of the population should be covered. Because the putative risks from inherent food plant toxicants are not zero, the risk should be fully described and, in most cases, provided in a range so that the risk manager can compare risks. This is particularly important at the international level where assessment advice is used for managing risks under varying economic and cultural conditions.

When the inherent food plant toxicant provides a beneficial effect at certain dietary levels, this should also be assessed, and a characterisation of risk/benefit should be provided. So, the strategy to avoid a priori any appreciable risk cannot be maintained for all inherent food plant toxicants. There is some similarity with nutrients.

For some nutrients the margin between the generally ingested amount or physiologically required dose and the maximal safe dose is narrow. For example, the amino acids methionine, phenylalanine and leucine are essential for body growth and maintenance, but in the rat the adverse effect level in mg/kg body weight is only 3–4 times the nutritional requirement in man (IFBC, 1990). Considering the essential amino acids only from a toxicological point of view, using a common safety factor of 100, or even of ten, would be detrimental for human nutrition. For vitamin A (retinol equivalent) the recommended intake in, potentially, pregnant women is 1 mg/day, the maximum safe level of daily intake is estimated at 3 mg, and teratogenic effects of retinol as vitamin A supplement have been observed at a daily intake of 7.5 mg/day (Rothman et al., 1995). The precise intake associated with abnormalities has not been defined (Oakley and Erickson, 1995; Mills et al., 1997). For energy intake the margin is possibly just a few percent. Over consumption of energy is considered to be the highest food born carcinogenic factor (National Research Council, 1996). Renwick (1991) calculated from literature the factor between normal human daily intake per body weight and the daily intake associated with toxicity for a number of food constituents. Application of usual uncertainty factors would result in levels that are inadequate for nutritional requirements.

The suspected carcinogenicity of selenium in animal experiments cannot result in regulatory actions for an absolute reduction of human exposure. Indeed, selenium is now considered to be an essential element and at low doses it even functions as an anti-carcinogen. So, for essential food constituents the simplified use of a safety factor is inappropriate: more toxicological, epidemiological and nutritional research is needed to reduce uncertainties where recommended intake levels and safety limits on both sides need to be balanced.

3.1. Matrix effects and interaction with other constituents

The bioavailability, kinetics and thus toxicity of the pure food plant toxicant or its active metabolite may be very different from when it is ingested in its common food matrix. Examples of such differences in bioactivity associated with different bioavailabilities due to matrix effects and by interaction with other food constituents are the following:

About 600 naturally occurring carotenoids are known. It has been found that the low and variable bioavailability of carotenoids from plant foods depends strongly upon the specific compound, the molecular linkage, the amount consumed in a meal, the matrix in which the carotenoid is incorporated, absorption modifiers, nutrient status of the host, genetic and other host related factors, and interactions between these factors (De Pee and West, 1996). The matrix in which β -carotene is embedded is a very important determinant of its bioavailability. β -Carotene may be organised in pigment-protein complexes which are located in cell chloroplasts, or it may be located in the chromoplasts of cells, often in lipid droplets. Cells of the plant first need to be disrupted in order to free β -carotene from its matrix. Secondly, the chloroplast or chromoplast needs to be accessed, and thirdly the β -carotene molecule, if bound, has to be separated from its ligand. Releasing β -carotene from a pigment-protein complex would be more difficult than freeing it from a lipid droplet (De Pee and West, 1996). Food processing, such as reducing particle size and heating, may reduce matrix effects and increase the bioavailability.

The cyanogen content of cassava flour in Africa usually grossly exceeds the safety limit set by the Codex Alimentarius (Essers, 1998), but toxic effects are rare under normal conditions. This can be explained partly because the cyanogens are mainly bound in glucosides which are relatively stable in the human body: in experiments with human volunteers less than 50% of the cyanogenic glucoside intake was found to be degraded to HCN (Carlsson et al., 1995). Also important is the form in which the product is consumed: cassava flour is prepared into a stiff paste, which forms an elastic ball in the stomach and reduces in size only very slowly.

This causes a slow release of the toxicant, which can then be detoxified more effectively by the body's defence mechanism than would occur after acute exposure (Schultz, 1984). Thus, the food matrix, which can be manipulated by processing and preparation, is an important factor in determining the bioavailability.

Indole I_3A , isolated from green cabbage, forms directly mutagenic *N*-nitroso compounds upon treatment with nitrite, but not when in the presence of the juice of green cabbage. In consequence, the contribution of I_3A to the total mutagenicity of nitrite treated Brassica was marginal (Tiedink, 1991). A recent review of epidemiological studies also suggested that consumption of Brassica vegetables is associated with a decreased risk of cancer, notably colon, rectum, stomach and lung cancer (Verhoeven et al., 1996). This net beneficial effect shows that the adverse effect of one component can be neutralised and even overruled by a positive effect from other components in the same plant food.

Heated apple juice contains both mutagenic and anti-mutagenic compounds, whereby the latter can neutralise the former ones. When fractionated, one fraction obtained from gel filtration of heated clear apple juice showed a dose-related mutagenic response, while whole heated clear apple juice did not. Apple juice samples caused a reduction in the mutagenic activity of the known mutagens NQO and MNNG in the Salmonella mutagenicity assay (Ekasari, 1989).

Based on mechanistic studies, carcinogenicity from nitrate plus amides, through conversion to nitrosamines, is suspected by some researchers. However, vitamin C present in vegetables, which are also a major dietary source of nitrate, has been shown to inhibit *N*-nitroso product formation by chemically reducing nitrous acid (Mirvish, 1983). Vegetable consumption is associated rather with a lower incidence of cancer (Steinmetz and Potter, 1991a,b).

In general, plant foods can be regarded as complex mixtures of which the composition varies between certain physiological limits. The adverse effect of one substance may be enhanced, supplemented or neutralised by others. The interaction of the plant toxicant with the food can be made by anti-oxidants, anti-mutagens, specific anti-toxicants, spatial or functional hindrance, or other factors that influence the kinetics of the ingested toxicant.

3.2. Balancing inherent food plant toxicants and health protecting constituents in food?

There is an argument for balancing the toxicant with the neutralising or otherwise health protective effect of the foodstuff in which the toxicant is contained. We suggest that this may only be considered when the protection factor is as inherent to the particular food as the toxicant and a reduced toxicity has been shown

either experimentally or by epidemiology. However, balancing the separate health effects of individual food constituents is a complex task. Apart from the immense number of constituents in food to balance, one cannot simply add known effects of each constituent in order to calculate a net health effect since this would omit any interactions that may take place.

Then **how** can one judge the quality of the plant-food product in relation to the presence of potential inherent food plant toxicants and of neutralising or otherwise health-promoting factors? Part of the solution might lie in attributing higher value to the toxicity (and testing) of the whole foodstuff, rather than the isolated substances. This more holistic approach is justified because the resultant toxicity of the foodstuff does not equal the sum of the effects of its constituents, and the number of constituents and possible influencing factors is too large to be studied individually. The suspected risk of single food plant toxicants should be verified by whole foodstuff testing. This will then help in establishing limits for specific constituents, if found necessary. Where cases of toxicity have been found with the whole food stuff, these data should be complemented by toxicodynamic and toxicokinetic data from studies of single substances and mixtures of some of the relevant constituents.

For subsequent risk management and regulation, consideration may be necessary between the benefits and adverse effects of alternatives, such as plant-contained inherent pesticides versus industrial pesticides, which probably can only be done on a case by case basis.

4. Regulatory aspects of inherent plant toxicants

4.1. Considerations from other institutions

The US Food and Drug Administration (FDA) utilises an approach for natural toxicants in 'traditional foods', based on the Food, Drug and Cosmetics (FD and C) Act, Section 402(a)(1), which is distinct from that for additives. If the poisonous or deleterious substance is a normal component of that food and not an 'added' substance, the food is prohibited from sale only if the quantity of such substance in the food 'ordinarily renders the food injurious to health' (Flamm et al., 1994). The US Congress instructed FDA to pay less attention to ingredients that had been in use for many years without observable adverse effects (Merrill, 1991). There appear to be two different safety standards, depending on whether the poisonous or deleterious substance is added or is a normal component of food. Traditional food was presumed to be safe, and for it to be found unsafe, it must have demonstrably affected the health of consumers of that food (Flamm et al.,

1994). This seems to imply 1) that the effect of possibly toxic substances in traditional foods should not be considered separately, but only in relation to the whole diet, and 2) that data are required from observational population studies.

As mentioned earlier, the US Committee on Comparative Toxicity of Naturally Occurring Carcinogens (National Research Council, 1996) concluded that both naturally occurring and synthetic chemicals can be evaluated by the same epidemiology or experimental methods and procedures. This seems to conflict with the implications of Section 402(a)(1).

Regarding regulation of naturally occurring toxins, the US FDA has interpreted FD and C Act Section 406 as permitting a balancing of the value of the food, the toxicity of the contaminant, and the feasibility of reducing or eliminating the contaminant. It concerns substances that have been historically acceptable to consumers, which often provide significant benefits, and are disproportionately difficult to avoid or remove (Ely, 1989).

4.2. Classification and priority setting of known inherent food plant toxicants

Plants are made up of many thousands of substances, most of which have not been subjected to toxicological research. An example of the vast number of bioactive substances in food plants is that 96 bioactive substances have already been identified in basil (Beier, 1990). The known inherent food plant toxicants can be classified in several categories, relating to their plant source, the chemical structure of the substance or its physiologically active metabolite, or to the mode of action. Overviews of known inherent food plant toxicants are given by Duke (1977), Liener (1980), Concon (1988), Cheeke (1989), Beier (1990), IFBC (1990), D'Mello et al. (1991), IPCS and ILSI Europe (1992). 1992 distinguished coumarins, cyanogens, cycasin, dioscorea alkaloids, furocoumarins, glucosinolates, glycoalkaloids, glyzrrhizin, haemagglutinins, hydrazines, hydrazones, isoflavones, lupin alkaloids, methylene dioxybenzenes, methylxanthines, pyrrolizidin alkaloids, saponins, tannins, toxic aminoacids, toxic fatty acids, vicine and convicine.

Priorities for research and evaluation of inherent plant toxicants have recently been proposed by IPCS and ILSI Europe (1992). The priority setting was based upon the following criteria:

1. The available toxicity data indicate chronic, rather than acute, effects.
2. High exposure to humans, including certain risk groups.
3. Insufficient experimental data and/or lack of adequate risk evaluation.

The regional importance of the crop or regional exposure levels are also used for establishing priorities.

Inherent toxicants from several plant source materials, including food plants, herbs and spices, are regulated according to the EU Council Directive on Flavourings (88/388/EU), eg. coumarin, safrole, thujonic and hydrocyanic acid (originating from cyanogenic glycosides). Very few other regulatory measures have been taken in this field. In contrast to food additives, there is hardly any direct economic stimulus to undertake comprehensive toxicological tests with inherent food plant toxicants. Speijers (1995) observed a vicious circle in the lack of data on their occurrence and toxicology: as long as nobody is made responsible for studying safety aspects of inherent plant toxicants, the lack of data will persist, and regulation based on thorough toxicological studies will remain absent. In rare instances, acute toxicity data are available from exposed humans (e.g. acute poisonings among mushroom-eaters). Observational epidemiological studies may provide data on long-term (chronic) health effects (such as the effects of xeno-oestrogens from dietary plants), which can be critically important for the risk assessment. However, in the majority of situations, direct human data are not available, and the potential risks must be predicted from experimental data.

To break the circle and make progress, Speijers suggests that at least some regulation is needed to create a demand for toxicological studies or to stimulate the quality of such studies. However, this may give another problem because even when data exist, it is often not clear on what grounds such regulation should be based.

Impetus towards regulatory action may be gained by the development of new biological breeding tools, which allow inherent food plant toxicant levels, as well as the factors that may modify their bioavailability, to be altered.

Examples of inherent food plant toxicants which have been subjected to some kind of regulation are the following.

4.2.1. *Solanum glycoalkaloids (SG)*

The product requirement for total glycoalkaloid content often found in literature is 200 mg/kg potato as 'safe limit' or 'upper safety limit' (Morris and Petermann, 1985). This may have been established on the basis of acute toxic effects being found in humans consuming potato solanine levels of 257 mg/kg and higher, and no acute toxic effects being seen after consumption of tubers with 196 mg/kg potato (Griebel, 1923; Bömer and Mattis, 1924, in: Van Gelder, 1989). Bömer and Mattis concluded that potatoes with a solanine content exceeding 200 mg/kg seem to cause adverse effects on human health. It is not clear whether the potatoes had been eaten with or without peel. Also the methodology for assessing glycoalkaloids in the old studies add uncertainty.

The Nordic Working Group on Food Toxicology and Risk Assessment (1991) suggested the following argument for regulation: The LOEL for SG in man is 2 mg/kg bw/day. Based on an estimated average daily intake of 300 g potatoes the limit of 200 mg/kg potatoes would correspond to 1 mg SG/kg bw/day in adults. Peeling causes two-thirds loss of SG, leading to 0.3 mg/kg bw/day. They suggest that a safety margin of a factor 2–6 exists, but also stress that higher potato consumption occurs, and that 8% of the early potatoes in Sweden contained > 200 mg/kg potato. They conclude that the safety margin for 'solanine' cannot be considered satisfactory. They recommend that 200 mg/kg should be the maximum acceptable level for potato varieties currently available on the Nordic market, and that efforts should be made to reduce the levels of SG; The SG levels in new potato varieties should not exceed 100 mg/kg.

In The Netherlands, the potato producers ('Commissie voor de samenstelling van de rassenlijst and Productschap voor Aardappelen') made an agreement ('convenant') among themselves to observe a limit of 100 mg/kg fresh weight for new potato cultivars (Bal, 1989).

4.2.2. *Pyrrolizidine alkaloids*

After an extensive study on pyrrolizidine alkaloids, IPCS (1988) concluded that 'Because of their known involvement in human poisoning and their possible carcinogenicity, exposure to pyrrolizidine alkaloids should be kept as low as practically achievable. The setting of regulatory tolerance levels for certain food products may be required in some situations.' The control of plant populations for preventing poisoning in man was carried out only in Uzbekistan, following the epidemics of human disease due to contamination of grain by seeds of *Heliotropium lasiocarpum* and *Trichodesma incanum*. In addition to several managerial measures, a state standard was set for the quality of grain stored for food. The limits of seeds of these two weeds were set at 0.2% and zero, respectively (IPCS, 1988).

4.2.3. *Cassava cyanogenic glucosides*

No safety limits have been established for fresh cassava roots, because the processing greatly influences the toxicant levels at consumption. For cassava flour a safety limit has been set by the Codex Alimentarius Commission of the FAO/WHO at 10 mg HCN/kg (CAC, 1991). The origin of this value is obscure. A literature review on studies of cyanogen levels in cassava products in Africa, indicated that none of the mean concentrations met with this requirement, which suggests that this limit is unrealistically low (Essers, 1998). If strictly adhered to, most of African cassava flour would probably be disqualified for consumption, thereby creating a famine of unprecedented size.

4.2.4. Glycyrrhizic acid in licorice

The critical effect of exposure to glycyrrhizic acid in licorice is inhibition of the enzyme 11-hydroxysteroid dehydrogenase which in turn leads to a syndrome of apparent hypermineralo-corticoid excess. Based on case reports and exposure of healthy volunteers, the Nordic Working group of Food Toxicology and Risk Assessment (NNT) established a provisional LOAEL of 100 mg/day for adults. It was recognised that symptoms may occur at even lower intakes in very sensitive individuals (Størmer et al., 1993). Since the effect of glycyrrhizic acid involves retention of sodium, a high intake combined with a high sodium intake as in our Western societies are probably not advisable. A recent study of the intake in the Dutch population showed that about 13% was regular users and the average intake of glycyrrhizic acid among these was 11 mg/day with a maximum of 188 mg. Of pregnant women 31% were regular users with a mean intake of 13 mg/day, the 95 percentile being 45 mg/day (Hulshof and Kistemaker, 1995). According to the guidelines of the EU Scientific Committee for Food (SCF) the intake of glycyrrhizic acid should not exceed 100 mg/day (Commission of the European Communities, 1992). This shows that the recommended maximum limit by the EU SCF is set at the LOAEL with no safety margins. Furthermore the safety margins of the high consumer group are also small.

Clearly, the few safety limits and product requirements that have been set for inherent food plant toxicants are not based on a NOAEL divided by an uncertainty factor, but are either somewhat above the maximal average ingestion (solanidine glycosides in potatoes), the LOAEL (glycyrrhizic acid), or even unrealistically low and therefore without compliance or effect (cyanogenic glucosides in cassava flour).

The legislation for the addition of new chemicals to foods is based on the proof that they are safe, with a sufficient safety margin to exclude any appreciable risk. In the case of inherent food plant toxicants in traditional foods, the substances are not new or alien to humans and so the effects, although possibly undesired or yet unknown, may not constitute new health hazards of an unknown magnitude. The safety testing of new chemicals prior to their approval, are designed to ensure that false negative results (when a substance is wrongly thought to be safe but in fact it is not) are excluded; that is, over-regulation is safer than under-regulation. With traditional foods which contain inherent food plant toxicants for which there is a history of apparently safe use, false positives (when a substance is wrongly thought to cause harm or risk of harm, but in fact it does not) should be avoided. Exceptions exist for proven highly toxic substances like pyrrolizidine alkaloids. It is widely accepted that the higher the suspected toxicity, and the more serious the effect, the less proof that is necessary before taking regulatory steps.

For the tolerance of long established foods with inherent toxicants, we suggest that the approach should therefore be the other way around to that outlined above: Enough evidence should be gathered to prove that the food product including that substance at a certain intake level gives rise to toxic effects, before regulation is effected which may endanger the production, trade or consumption of current products. The question of what is 'enough evidence' then becomes the crucial issue, to be judged by expert committees.

5. Risk estimation of inherent food plant toxicants

To assess the risk of inherent food plant toxicants, a number of aspects which are more or less characteristic to these food plants, has to be taken into consideration.

5.1. Incorporating human experience

How can the history of virtually or apparently safe use be incorporated in risk assessment? Information on consumer morbidity and mortality associated with the use of the product could be related to the number of years that the product has been used, the number of people that consume the product regularly, the availability and outcome of medical and epidemiological data and, where possible, linked with the levels of the toxicant at the moment of safe consumption and of toxic effects. A 'human experience factor' might possibly be expressed quantitatively and used as an alternative to the uncertainty factor for the human heterogeneity. The logic behind this is that the more people who have used the product without ill effects for a longer time, and the better this has been studied, the lower the uncertainty factor. The viability of this concept should be examined and illustrated by examples. A first effort to apply it to solanum glycoalkaloids did not lead to useful results for the following reasons:

1. Cases of known poisonings were often due to unusual circumstances, such as consumption of peel, sprouts, immature or green potatoes;
2. Detailed exposure data on quantity and kind of glycoalkaloids were usually lacking;
3. It is not known which part of common gastro-intestinal disturbances in the population are related to potato consumption.

Another option is *not* to integrate this information as a factor in a formula based on toxicological studies, but to keep it separate next to the calculated risk, and balance them, as has been done so far implicitly. The criticism of ad hoc or even post hoc regulation would not be obviated unless this balancing is made according to clearly defined rules. However, it is not yet clear if uniform rules will be applicable in a meaningful and exhaustive way. Therefore, examples are necessary.

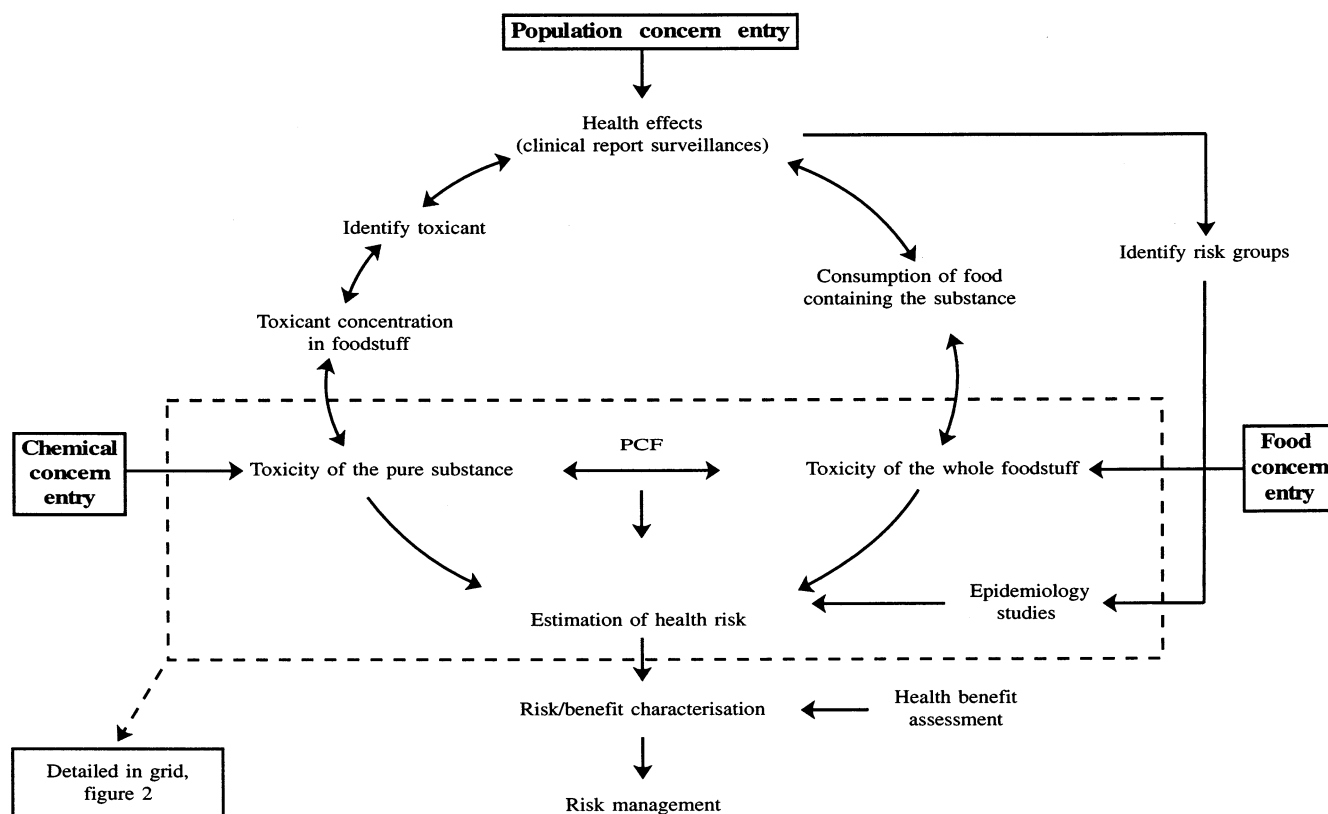


Fig. 1. A test system for risk management of plant foods with inherent toxicants.

Apart from using general human experience, detailed epidemiological studies and toxicological testing remain necessary for suspected food constituents. No single test system provides sufficient information either to describe the health characteristics or to assess the risk of an inherent food plant toxicant. A combination of tests is necessary, including tests of the single substance under study, as well as the whole foodstuff which contains the toxicant(s). This approach is visualised in Fig. 1.

The kind of the initial research depends on whether it is triggered by health effects in the population, by concern on properties of a chemical or the food, and on the available data, and the kind and severity of the toxic response.

For example, in cases of population concern from reported health effects associated with consumption of a foodstuff, it is logical to start research by studies on the consumption pattern of the foodstuff associated with health problems, possibly followed by (animal) toxicity testing of the whole food. Another route may be the identification of the toxicant, estimation of its concentration in foodstuff and toxicity testing of the pure substance followed by estimation of health risk based on food consumption and toxicity of the pure compound. Comparison between the toxicity of the foodstuff and the pure compound results in establishment of the product correction factor (PCF).

A third route contributing to the estimation of health risk comprises epidemiological studies, including identification of risk groups.

The relationship between toxicity of the pure substance and that of the whole foodstuff in animals and humans is described in paragraphs 5.3 and 5.4 and visualised in Fig. 2.

5.2. Whole foodstuff testing

The critical issue in risk assessment is the toxicity of the whole diet in relation to the susceptibility of the consumer. As consumers compose their diet of different foodstuffs, which therefore may appear in different combinations, the basic unit to test for toxicity is the processed and prepared whole foodstuff. It implies an intrinsic balancing of the amount of toxic substances and of factors modifying the toxic responses, such as matrix and interacting constituents². It can be achieved by substituting part of the diet by the food to be tested, and compensating for energy and (micro-)nutrients. The proportion of the food in the diet then depends on its usual place in the diet, e.g. for potatoes this might

² This corresponds with the EPA Guidelines for the Health Risk Assessment of Chemical Mixtures (1986), which states that the carcinogenic effects of a mixture can best be examined by testing the mixture.

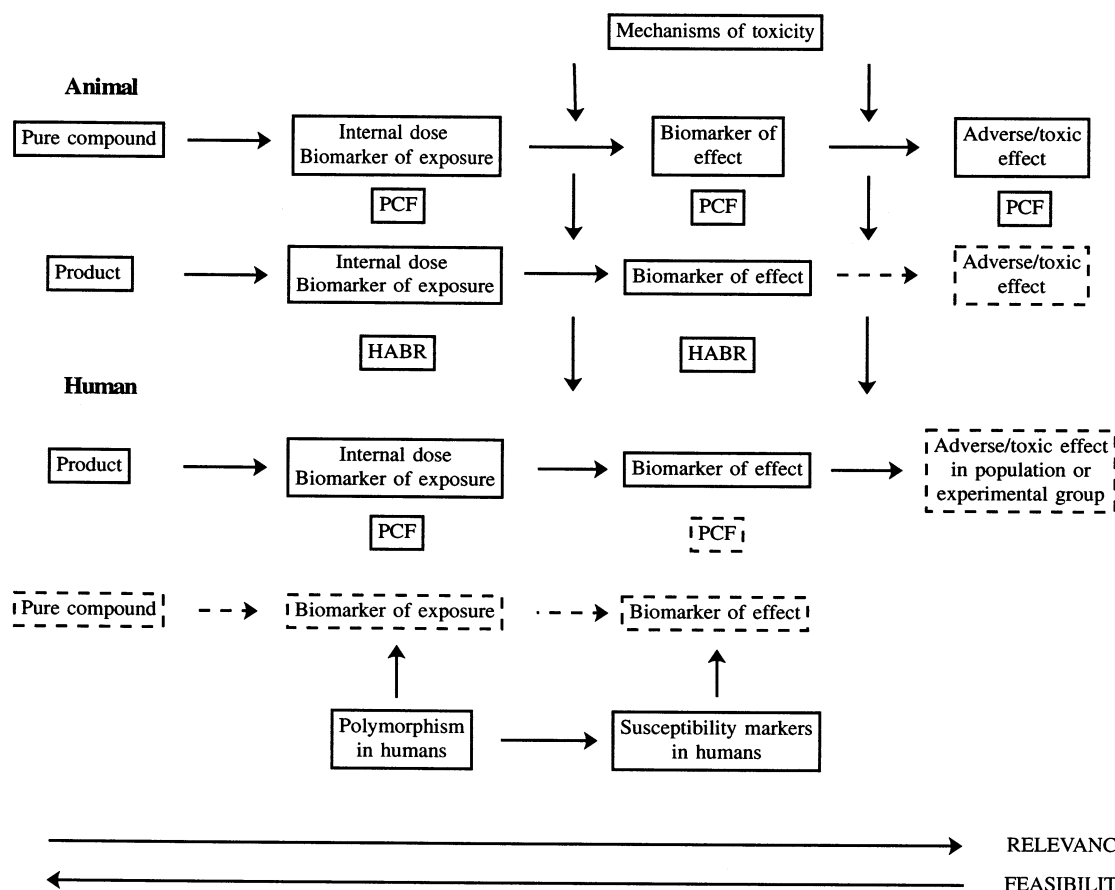


Fig. 2. A scheme of animal and human testing, arranged as an interactive grid for assessing the relationship between the toxicity of pure substances and that of the whole food in which the substances occur. Indicated is the relevance of the data and the feasibility of obtaining them.

range up to 60% energy, for leafy vegetables up to 10 g/kg body weight, and for black pepper up to some 300 mg/kg body weight. An example of this approach is the study of the long-term wholesomeness of autoclaved or irradiated pork in rats by Van Logten et al. (1983), in which 35 w/w % of the diet was replaced with treated pork. Another example can be seen in the chronic studies with complete human diets in rats by Alink et al. (1989, 1993). The diets were composed according to mean consumption figures in the Netherlands.

Experimental studies with human volunteers are especially relevant and because the toxicants and the food are not new or alien, such studies do not raise significant problems for ethical considerations. Studies using human volunteers have the advantage that no extrapolation from animals to humans is necessary and that certain aspects can be investigated, such as the effects on higher brain functions (mood, headache), which cannot be studied in animals. Toxicokinetics can be studied also and then compared with those from animal models.

Epidemiological studies among humans can consist of observational studies relating exposure (intake levels or biomarkers of exposure) to health effects (e.g. case-

control or cohort studies), and of randomised controlled trials that are used for testing potentially beneficial foods or ingredients on disease endpoints. Examples are the β -carotene and lung cancer trial in Finland, and the US Women's Health Trial on effect of fat reduction and increased vegetables/fruit consumption on breast cancer. However, randomised controlled trials using hard disease endpoints with potential toxicants are not feasible or ethical. In those instances, in humans only studies with biomarkers of exposure and effect, as well as reversible physiological effects, are feasible. The development and use of biomarkers is crucial to increase the sensitivity of the human and animal assays.

Historically, the selection of plants for human consumption has been based on the safety of the *whole plant food*. If the plant was found to be toxic, it might still have been consumed if adequate processing eliminated or deactivated the toxic factor. During the development of toxicology, emphasis was put on isolation, characterisation and quantification of the toxic principle and dose/effect relationships. This is still important. However, for inherent toxicants in plant foods not only does the toxic principle and its dose determine the

toxicity, but other food factors can alter the bioavailability of the reactive metabolite or give rise to interactions such as synergism and antagonism. Therefore, the toxic effect of the whole foodstuff has to be taken into account.

5.3. Toxicity characterisation

Ideally the whole foodstuff should be tested in both animal and human subjects. In practice this will not always be possible. Thus the amount of the whole foodstuff that can be consumed by experimental animals is constrained by the need to feed nutritionally balanced diets and the concentration of the toxicant in the foodstuff may be too low to induce adverse effects in animals. In feeding trials with human subjects the possible consequences of the toxicity may prevent experimental testing.

A solution to this problem is to organise the variables characterising toxicity into a grid in which their inter-relationships are displayed. The incorporation of appropriate data into this grid allows a quantified assessment to be made of the probable range of the toxic effect of the foodstuff on human subjects. The grid for toxicity characterisation is shown in Fig. 2.

The grid is formed from two strata, animal and human studies interacting with the degree of exposure and the magnitude of the effect. Each stratum consists of two parts, the pure substance and the whole foodstuff, described as the product. The evaluation of toxicity of a foodstuff progresses across the grid from left to right. The direction is from the most readily established data with the isolated toxicant in animals towards the determination of the toxicity of the whole foodstuff, the product, in humans.

The pure substance and the foodstuff in which it occurs, the product, are each evaluated in animals and humans so far as ethical and practical constraints permit. Progressing across the grid, the challenges are to establish a measure of exposure and to observe, define and quantify the toxic effect.

Direct quantification of the toxic effect of the food-contained toxicant may not be possible, even in animals, and consequently markers of exposure and of effect are used.

Biomarkers may be:

1. *Exposure biomarkers*, which relate the internal dose, or target organ dose, to the external dose and are specific for that chemical and animal model.
2. *Effect biomarkers*, which relate the effect, or a surrogate endpoint, to the external dose, and may be either specific to the chemical or non-specific.
3. *Susceptibility biomarkers*, which relate to the differences in susceptibility of exposed individuals, for example a genetic polymorphism. Such biomarkers are of greatest value in interpretation of epidemiological studies.

The degree of uncertainty associated with the final evaluation of the toxicity of the product is least when the greatest number of the possible considerations in the grid have been taken into account.

There are many risk assessment scenarios. For example, the toxic effect may have been established with the isolated toxicant in the animal studies, while it may not be possible to feed sufficient quantity of the product to achieve a toxic effect. Risk assessment is possible providing that exposure to the product has been defined and its effect monitored through biomarkers. The use of biomarkers of exposure makes it possible to predict the extent of any adverse toxic effect of the product in the animal.

The quality of this quantified prediction is primarily dependent on the quality of the experimental data. The use of the data is enhanced by the selection of appropriate sections of the grid for interaction, and by quantifying the interaction by the establishment of ratios.

The following factors for the risk estimate of inherent food plant toxicants are proposed: product correction factor (PCF), relating data on the product (food) to the pure chemical within the animal stratum or within the human stratum; and human-animal biomarker ratio (HABR) to interrelate the animal and human strata. These factors are described below.

5.4. Product correction factor

Each of the animal and human sets of data (Fig. 2) includes assessments of the toxicant in isolation and of the foodstuff containing the toxicant. The comparison of biomarkers of exposure and/or for effect of the food-contained toxicant and for the toxicant in pure form is considered fundamental in this approach. In this way, the alterations in toxicity due to the foodstuff will be evident. The comparison of foodstuff and isolated toxicant can be combined to provide a correction factor. The correction factor is a property of the foodstuff that quantifies its capacity to modify the response to the toxicant. We call this factor the 'product correction factor' (PCF). It is determined by the ratio of the quantity of the food-contained substance and the quantity of the pure substance which gives rise to the same biomarker and/or toxic effect in the same animal model or in humans.

$$\text{PCF} = \frac{\text{dose/effect} \times \text{phytotoxin in food}}{\text{dose/effect} \times \text{pure phytotoxin}}$$

Depending on dose level, choice of effect and severity of effect, the PCF may vary. The displacement and the steepness of the curve for the food-contained toxicant (with respect to the curve of the pure chemical) describe the capacity of the whole foodstuff to modify the toxic response and will depend on the foodstuff composition. It is possible that the dose-effect curves for the pure

substance and for the whole food product do not run parallel. This may occur, if a low toxicant level is completely neutralised by other constituents in the food, while a high concentration of toxicant in the food is only partially neutralised by the then limited amount of other constituents. The possible complex character of this quotient is exemplified in Fig. 3. The relevant PCF then is the ratio of values of the curve which relate to the highest no-effect level or to the highest actual product and uptake levels.

The effect/dose may be the LOAEL or the NOAEL or, if toxicological effects cannot be elicited, the critical biomarker may be taken as an effect. In a simplified approach, the factor can be defined as the ratio of the LOAEL of the substance in food to the LOAEL of the pure substance:

$$\text{PCF} = \frac{\text{LOAEL}_{\text{phytotoxin in food}}}{\text{LOAEL}_{\text{pure phytotoxin}}}$$

The PCF is 1 if there is no additional net effect of the foodstuff; the PCF is < 1 if there is synergism or addition between the toxic substance and other substances; the PCF is > 1 if the food contains health protecting principles which diminish the effect of the toxic principle. This may be due to altered bioavailability (e.g. matrix effects) or interaction with other substances (e.g. antioxidants) at the toxicokinetic or toxicodynamic phase resulting in protective effects.

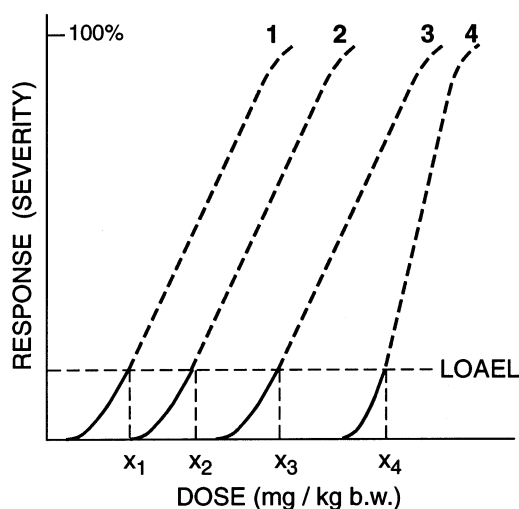


Fig. 3. Possible (hypothetical) dose/response curves for pure (2) and food-contained inherent (1,3,4) toxicants 1) Food contained toxicant. PCF < 1 due to synergism from the food, or inhibition of detoxification in the body. 2) Pure toxicant. 3) Food contained toxicant. PCF > 1 due to antagonism from the food, or inhibition of activation in the body. 4) Food contained toxicant. PCF > 1 due to dose dependent interaction or saturation of metabolism. The Product Correction Factor (PCF) is the ratio of a dose at a given response in curve 1, 3 or 4, divided by the dose of curve 2 at the same response. Only the lower part of the curve (up to the LOAEL) is of interest. Although curve 4 does not parallel that of the pure compound, the PCF can still be established as e.g. $X_4 : X_2$.

If a toxic effect cannot be elicited in the whole-food-stuff experiment, a specific biomarker, related to the toxic action of the substance can be used as an end-point. It is recognised that a suitable biomarker of effect may not be available especially to predict chronic toxicity. Biomarkers of exposure are in principle easier to be measured and can be used to detect possible changes in bioavailability and possible metabolism of the chemical due to the other food constituents. It is important to obtain mechanistic information from animal experiments and in vitro studies in order to identify suitable biomarkers and to improve the interpretation of experimental results. It is also important to conduct animal and human studies to assess the predictive ability of biomarkers regarding hard disease endpoints, so as to define their suitability.

Knowing this relationship makes the use of a wide range of techniques and data possible: a small database of foodstuffs can be linked to a larger database of pure substances. This enables predictions to be made for other constituents in the same foodstuff and for similar constituents in other foodstuffs. The PCF will be a tool for risk managers to apply the information developed for toxicants in isolation to food-contained toxicants. It may provide information for food industries to further reduce risks from inherent food plant toxicants.

5.5. Animal to human extrapolation

Observations of toxic effects in humans, related to a specific toxicant in food, are often not available and it is not possible or desirable to induce toxic effects with experimental feeding of the whole foodstuff, in humans. In the absence of human effect data, interspecies comparisons can be made on the basis of selected biomarkers. A similar mechanism of toxicity must be presumed in animals and humans, but this is inherent to any risk assessment based on animal studies.

For the comparison of biomarker levels in animals and humans we propose the term 'human-animal biomarker ratio' (HABR). The HABR is the ratio of the dose-related biomarker levels in humans and in animals. Ideally, specific effect biomarkers should be used to derive HABR's, but if this is not possible, biomarkers of exposure may yield some useful information for the extrapolation from animals to humans.

Extrapolation to estimate toxic levels in humans may be feasible, based on the data observed from the animal model representing an extended dose-range and from the human studies obtained at relatively low dose ranges where only changes in biomarkers would be observed. This extrapolation is only valid if the animal and human response curves are assumed to be similar. Depending on the type of association between the selected biomarker and the predicted toxic effects, a margin of uncertainty may exist.

$$\text{Human – Animal Biomarker Ratio} = \frac{[Bh]/\text{Dose } h}{[Ba]/\text{Dose } a}$$

[Bh] = Biomarker's level in humans (e.g. in nmol/ml serum). [Ba] = Biomarker's level in animals (in same units). Dose h = dose of substance (e.g. in mg/kg body weight), in humans. Dose a = dose of substance (in same units), in animal model.

5.6. Risk assessment of inherent food plant toxicants requires epidemiology as well as toxicology

Toxicological tests are necessary for a proper assessment of inherent toxicity of food products. They may elucidate whether a product is as safe as presumed, identify toxic responses, discern the toxic constituents, discover dose-effect relationships, and establish kinetics and mechanisms of action. Experimental toxicology is very useful for the prediction of potential health hazards. With the testing of pure chemicals the test dose can be increased by a factor ten or more to provoke, reveal or enhance any effect and to trigger certain effects in a larger group of the test population. We have already discussed that it is necessary to test not only the pure substance, but also the whole foodstuff. However, levels of inherent food plant toxicants in common human food items are, in general, below those which cause observable adverse effects. Toxicological testing of inherent food plant toxicants at such low doses in groups of animals or humans may not yield useful information, whilst at the human population level such doses might still lead to toxic effects which are very mild, occur after some time or which have a low statistical probability or incidence, such as cancer and allergenicity. If the highest possible physiological intake levels of the whole foodstuff are close to the levels actually consumed, as with staple foods, then experimental tests with human volunteers will not cover the heterogeneity of the whole population with regard to human susceptibility and diet.

As discussed earlier, using an uncertainty factor to bridge the gap between studies with a small number of humans and a human population at large would be inappropriate. Vainio et al. (1990) (IARC) concluded that the most relevant quantitative data for making risk estimates for complex mixtures come from epidemiologic studies of populations exposed to the complete mixture.

Bearing in mind the adverse effects of the pure substance found in toxicological tests, cases of poisoning in the past, if available, and different populations consuming different quantities of the food and the toxicant should be investigated to assess possible relationships with their health status and health history. From such data it may be possible to derive levels of 'apparently safe' use and thus include the human experience

to complement toxicological evidence. So, in case of inherent food plant toxicants which rarely give rise to toxic effects or only to very mild effects in humans, we also have to rely on epidemiological data for a proper risk assessment.

Basing assessments on epidemiological data is not new. The National Health Council of The Netherlands (1985) and the JECFA (IPCS, 1987) differentiate between ADI values on the basis of experimental animal studies (in which a safety factor of 100 is used) and based on epidemiologic data of oral intake (to which a safety factor of 10 is applied). The National Health Council of The Netherlands (1994) states that for the risk assessment of carcinogens adequate epidemiological data should prevail over animal data; it adds, however, that such solid data are rare. In occupational risk assessment, where relatively well defined conditions prevail and effects are known, it is common that the human experience data are used as the departure point for risk assessment.

However, epidemiological studies frequently suffer from imprecise characterisation of exposure and/or health effects. Also, the number of confounding factors in assessing the health implications of common foods in a population can be large, and risk assessment is usually based on toxicological studies, although these suffer from the problems of high to low dose extrapolation and species extrapolation from animals to humans.

Therefore: epidemiological studies may be important in determining whether a toxic constituent produces adverse effects in human reality, while toxicological tests are necessary for assessing which effects might occur (hazard identification) and mechanisms of toxicity. Together epidemiological and toxicological studies may reveal whether health effects found at the population level are likely to be caused by a certain foodstuff or constituent of it. The use and integration of several disciplines is essential for the risk assessment of inherent food plant toxicants in traditional products (Table 1): Usually, there is first some evidence that toxic effects are possibly related to a food product or a food constituent from clinical reports on cases of poisoning (medical case studies), possibly followed up by observational population studies. This may trigger both experimental toxicological and epidemiological studies. The use of biomarker-based epidemiological studies may prove to be of particular importance. Studies in other disciplines are necessary for understanding the conditions which may lead to toxic effects, for measuring the toxicant and for developing techniques to reduce or control the risks.

An example of this multidisciplinary approach is the research on the outbreak of an unknown paralytic disease 'konzo' in a rural area in Mozambique. A large epidemiological survey was carried out, which included physical, neurological and nutritional parameters (Ministry of Health of Mozambique, 1984). This research

Table 1

Disciplines involved in risk assessment on intrinsic plant food toxicants

Toxicological testing of the (suspected) pure component

Assess whether the effect found in humans can be repeated experimentally;
 Assess possible toxic effects, the critical effect, and establish a dose-effect relationship;
 Assess the mechanisms of the toxic action;
 Assess exposure and effect biomarkers;
 Assess the possible kinetics and bioavailability of the pure substance (absorption, resorption, distribution, biotransformation and elimination).

Toxicological testing of the integral food product

Compare the results of the kinetics, toxic effects and dose-effect relationship with those found for the pure component.

Exposure and intake assessment

Qualitatively and quantitatively assess the relevant (toxic) constituents, localization in the product, their stability etc. and the effect of different processing techniques.
 Assess in the population the consumption of the foodstuff and calculate the intake of the constituent in different categories of consumers.
 Identify agronomic factors that influence phytotoxin levels.

Epidemiology and case reports

Problem signalizing: Information from population studies and case studies. Epidemiology can play a role especially in identifying the chronic, cumulative and teratogenic effects, and minor acute or semi chronic effects which have a low statistic probability. Detailed studies may point to either whole foodstuffs or constituents.
 Verifying in the population certain effects found in toxicological experiments.
 Establishing the magnitude of a toxicity problem.
 Assess the adverse health effects as well as the nutritional benefits of the foodstuff.
 Estimate the history of safe use and toxic effects in the past and describe it in terms of 'human experience', also indicating vulnerable categories of consumers.

These steps do not necessarily have to be followed in the order given, and often show a cyclic sequence: advances in one discipline provoke more refined research in another.

was later extended to several other geographical areas. These studies identified, described and defined the disease; a statistical relationship with cyanide exposure from the consumption of insufficiently processed cassava roots of high cyanogenic varieties was shown (Howlett et al., 1992; Tylleskär, 1994), and factors that had led to this consumption and the insufficient processing were revealed (Ministry of Health of Mozambique, 1984; Essers et al., 1992). Animal studies had shown the metabolic routes of the toxicant. Assessment of the toxicants in the food and extremely elevated levels of their exposure biomarkers in new konzo victims and not in controls further supported a causative role of the toxicant (Rosling, 1994). Feeding studies with human volunteers subsequently yielded some information on the kinetics of the food-contained toxicants in humans (Carlsson et al., 1995).

Animal experiments have yet to be undertaken to provide evidence that the paralytic disease can be elicited experimentally, to show which metabolic routes and which co-factors are essential, and to elucidate the mechanisms leading to the neurological damage. Additional studies on cyanogen exposure and (absence of) health effects in cassava consuming populations may lead to realistic risk estimates.

5.7. Establishing priorities for future study

Establishing priorities for deciding which inherent food plant toxicants to study should be based on the severity of the (estimated) health risk involved, the extent and intensity of human exposure; other common criteria include the feasibility of obtaining useful results. For risk management and regulation the possibilities of minimising the risk and the related economic burden for society is also taken into account. The predicted population burden of the health hazard is a product of the actual or potential health effect, exposure (actual or expected consumption and uptake levels and forms) and the number and demography of those exposed. As the actual effect of certain inherent food plant toxicants on human health is usually unknown when priorities are established, hypothetical extrapolations on the basis of known or expected effects can be useful, e.g. when a toxicant resembles or is part of a group of known toxicants. In novel foods, certain suspected chemicals may be in higher concentrations than usual, or new entities might be formed, or the concentrations of neutralising factors may be reduced, which may lead to research priority.

An obvious and essential criterion for establishing priority in research where each of these considerations is concerned is that there is insufficient experimental data or a lack of adequate risk evaluation.

The development of relevant scientific data, such as bioavailability, Pharmacologically Based Pharmacokinetic (PB-PK) modelling, mechanistic data and PCF, is important for the development of scientifically defensible risk assessments. This applies equally to the traditional approaches (for example replacing default uncertainty factors by data-derived values for threshold effects and PB-PK modelling of target organ doses for genotoxic carcinogens) and to the procedures for inherent food plant toxicants described in this paper. In the absence of appropriate data, risk assessments normally adopt conservative procedures. The characterisation of a clear health benefit of the *foodstuff* should be the rationale for the adoption of a risk assessment which is less conservative than the safety evaluation which would be applied to a food additive or environmental contaminant.

6. Recommendations

In the light of the small safety margins for many inherent toxicants in plant food, there is a need for more accurate risk assessments of these substances in food. There is very little data on these substances. In order to perform such risk assessments, more data will be needed.

It is necessary to establish an information system (data base) containing critically assessed data on composition and toxicological potential of inherent toxicants and protective factors in food plants and products thereof.

For risk assessment of inherent food plant toxicants, it is essential to consider the whole foodstuff and to take into account the action of protective factors and possible interactions between the various food constituents.

Data on adverse effects and biomarkers of effect and exposure are necessary for risk assessment of inherent food plant toxicants.

The procedure proposed here for risk assessment of inherent food plant toxicants, allows the combination of data from animal and human studies to provide a scientifically based risk assessment.

Adoption of the procedures described in this paper represents an important step in the establishment of scientifically based risk assessment for inherent food plant toxicants.

Acknowledgements

This paper is the outcome of the meeting with the same name, held from October 8–10, 1997, in Wageningen, The Netherlands, under the auspices of the European Training and Assessment Foundation (ETAF), Wageningen, The Netherlands.

References

- Adlercreutz, H., Mazur, W., 1997. Phyto-oestrogens and Western diseases. *Ann. Med.* 29, 95–120.
- Alink, G.M., Kuiper, H.A., Beems, R.B., Koeman, J.H., 1989. A study on the carcinogenicity of human diets in rats: The influence of heating and the addition of vegetables and fruit. *Food Chem. Toxicol.* 27 (7), 427–436.
- Alink, G.M., Kuiper, H.A., Hollanders, V.M.H., Koeman, J.H., 1993. Effect of heat processing and of vegetables and fruit in human diets on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. *Carcinogenesis* 14 (3), 519–524.
- Bal, A., 1989. Visie van het Ministerie van Landbouw en Visserij op natuurlijke toxinen. [Vision of the Netherlands Ministry of Agriculture and Fisheries on natural toxins] *De Ware(n)-Chemicus* 19 (November), 149–151.
- Barnes, D.G., Daston, G.P., Evans, J.S., Jarabek, A.M., Kavlock, R.J., Kimmel, C.A., Park, C., Spitzer, H.L., 1995. Benchmark dose workshop: Criteria for use of a benchmark dose to estimate a reference dose. *Regul. Toxicol. Pharmacol.* 21, 296–306.
- Beier, R.C., 1990. Natural pesticides and bioactive components in foods. *Rev. Environ. Contam. Toxicol.* 113, 48–137.
- Bömer, A., Mattis, H., 1924. Der Solaniningehalt der Kartoffeln. *Z. unters. Nahr-Genussm.* 47, 97–127.
- CAC, 1991. Codex Alimentarius, vol. XII, suppl. 4. Codex Standard for edible cassava flour (African Regional Standard). Rome, Food and Agriculture Organization of the United Nations.
- Carlsson, L., Mlingi, N., Ronquist, G., Rosling, H., 1995. A specific and sensitive method for determination of linamarin in urine. *Natural Toxins* 3, 378–382.
- Cheeke, P.R. (Ed.), 1989. *Toxicants of Plant Origin*. CRC Press, Boca Raton, FL.
- Commission of the European Communities, 1992. Food science and techniques. Reports of the Scientific Committee for Food (twenty ninth series). Recommendation on glycyrrhizic acid in liquorice products. Opinion expressed on 11 October 1991. Report EUR 14482 EN, Brussel.
- Concon, J.M. (Ed.), 1988. *Food Toxicology*. Marcel Dekker, New York.
- De Flora, S., Zanacchi, P., Izzotti, A., Hayatsu, H., 1991. Mechanisms of food-borne inhibitors of genotoxicity relevant to cancer prevention. In: Hayatsu, H. (Ed.), *Mutagens in Food. Detection and Prevention*. CRC Press, Boca Raton, FL, pp. 157–180.
- Deschner, E.E., Ruperto, J., Wong, G., L Newmark, H., 1991. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis* 12, 1193–1196.
- De Pee, S., West, C.E., 1996. Dietary carotenoids and their role in combatting vitamin A deficiency: Review of the literature. *Eur. J. Clin. Nutr.* 50 (Suppl. 3), S38–S53.
- D'Mello, J.P.F., Duffus, C.M., Duffus, J.H., 1991. Toxic Substances in Crop Plants. The Royal Society of Chemistry, London, p. 339.
- Duke, J.A., 1977. Phytotoxin tables. *CRC, Critical Reviews November*, 189–237.
- Ekasari, I., 1989. The inner quality of orange juice and apple juice-assessment by the use of microbiological methods. Thesis, Wageningen Agricultural University, Wageningen, The Netherlands. p. 120.
- Ely, C. Jr., 1989. Regulatory distinctions between naturally occurring and added substances in food. In: Taylor, S.Z., Scanlan, R.A. (Eds.), *Basic Symposium Series: Food Toxicology, a Perspective on the Relative Risks*. Dekker, New York.
- Essers, A.J.A. 1998. Risk assessment of cassava cyanogens. In: Gry, J., et al. (Eds.), *Information on inherent food plant toxicants. Guide to resources generated by the EU-AIR-NETTOX project*. Danish Veterinary and Food Administration, Mørkøhøj Bygade 19, 2800 Søborg, Denmark (in press).
- Essers, A.J.A., Alsén, P., Rosling, H., 1992. Insufficient processing of cassava induced acute intoxications and the paralytic disease konzo in a rural area of Mozambique. *Ecol. Food Nutr.* 27, 17–27.
- FAO/WHO, 1958, Procedures for the testing of intentional food additives to establish their safety for use. Second report of the Joint FAO/WHO Expert Committee on Food Additives. FAO Nutrition Meetings Report Series No. 17, Rome.
- FAO/WHO, 1991. Evaluation of certain veterinary drug residues in food. Thirty-eight report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, No. 815, Geneva.
- Flamm, W.G., Kotsonis, F.N., Hjelle, J.J. 1994. Threshold of Regulation: a unifying concept in food safety assessment. In: Kotsonis, F.N., Mackay, M., Hjelle, J.J. (Eds.), *Nutritional Toxicology*. Raven Press, New York, pp. 223–233.
- Griebel, C., 1923. Solaninreiche gesundheits—Schädliche Kartoffeln. *Z. Unters. Nahr-Genussm.* 45, 175–183.

- Gry, J., Kovatsis, A., Rhodes, M., Rosa, E., Rosner, H., Speijers, G., Soborg, I., Walker, A., 1998. Information on inherent food plant toxicants—Guide to resources generated by the EU-AIR-NET-TOX project. Danish Veterinary and Food Administration, Mørkøj Bygade 19, Søborg, Denmark (in press).
- Harborne, J.B., 1988. Introduction to Ecological Biochemistry, 3rd edn. Academic Press, New York, p. 356.
- Heaney, R.K., Fenwick, G.R., 1995. Natural toxins and protective factors in Brassica species, including rapeseed. *Nat. Toxins* 3, 233–237.
- Hertog, M.G.L., 1994. Flavonols and Flavones in Foods and their Relation with Cancer and Coronary Heart Disease Risk. PhD Thesis, Lanbouuniversiteit, Wageningen, The Netherlands, p. 151.
- Howlett, W.P., Brubaker, G., Mlingi, N., Rosling, H., 1992. A geographical cluster in Tanzania. *J. Trop. Geographical Neurol.* 2, 102–108.
- Hulshof, K.F.A.M., Kistemaker, C., 1995. The consumption of licorice and the intake of glycyrrhizic acid among Dutch population groups. TNO report No. V 94.599. The Netherlands.
- IFBC, 1990. Biotechnologies and food: Assuring the safety of foods produced by genetic modification. *Regul. Toxicol. Pharmacol.* 12 (3), 11–78.
- IPCS, 1987. Principles for the safety assessment of food additives and contaminants in food. *Environmental Health Criteria* 70. WHO, Geneva.
- IPCS, 1988. Pyrrolizidine Alkaloids. *Environmental Health Criteria* 80. WHO, Geneva. p. 345
- IPCS, 1994. Assessing human health risks of chemicals: derivation of guidance values for health based exposure limits. *Environmental Health Criteria* 170. WHO, Geneva.
- IPCS and ILSI Europe, 1992. Final report of the steering group on naturally occurring toxins of plant origin, Carshalton, UK, 20–22 May 1992. *IPCS, WHO/PCS/92.69*.
- Koratkar, R., Rao, A.V., 1997. Effect of soya bean saponins on azoxymethane-induced preneoplastic lesions in the colon of mice. *Nutr. Cancer* 27, 206–209.
- Lamartiniere, C.A., Moore, J., Holland, M., Barnes, S., 1995. Neonatal genistein chemoprevents mammary cancer. *Proc. Soc. Exp. Biol. Med.* 208 (1), 120–123.
- Liener, I.E., 1980. Toxic constituents of plant foodstuffs. Food science and technology—A series of monographs, 2nd edn. Academic Press, New York. p. 502.
- Medlock, K.L., Branham, W.S., Sheehan, D.M., 1995. Effects of coumestrol and equol on the developing reproductive tract of the rat. *Proc. Soc. Exp. Biol. Med.* 208 (1), 67–71.
- Merrill, R.A., 1991. Regulatory toxicology. In: Amdur, M.O., Doull, J., Klaassen, C.D. (Eds.), *Casarett and Doull's Toxicology—The Basic Science of Poisons* 4th edn. Pergamon, Oxford, pp. 970–984.
- Messina, 1992. Phytate's potential role in reducing cancer risk. *American Journal of Clinical Nutrition* 55, 762.
- Mills, J.L., Simpson, J.L., Cunningham, G.C., Conley M.R., Rhoads, G.G. 1997. Vitamin A and birth defects. *Am. J. Obstet. Gynecol.* 177, 31–36.
- Mirvish, S.S., 1983. The etiology of gastric cancer: intra-gastric nitrosamine formation and other theories. *J. Natl. Cancer Inst.* 71, 629–647.
- Ministry of Health of Mozambique, 1984. Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area in Mozambique. *Bull. WHO* 62, 477–492.
- Morris, S.C., Petermann, J.B., 1985. Genetic and environmental effects on levels of glycoalkaloids in cultivars of potato. *Food Chem.* 18, 271–282.
- National Health Council of The Netherlands, 1985. Advies inzake uitgangspunten voor normstelling. Ministry of Health, The Hague, The Netherlands.
- National Health Council of The Netherlands, 1994. Risk assessment of Carcinogenic chemicals in The Netherlands. *Regulat. Toxicol. Pharmacol.* 19, 14–30.
- National Research Council, 1996. Carcinogens and Anticarcinogens in the Human Diet. National Academy Press, Washington, p. 10.
- Nordic Working Group on Food Toxicology and Risk Assessment, 1991. Food and New Biotechnology. Novelty, Safety and Control Aspects of Foods Made by New Biotechnology. Nordic Council of Ministers, Nord, p. 18.
- Oakley, G.P., Erickson, J.D., 1995. Vitamin A and birth defects. Continuing caution is needed. *N. Engl. J. Med.* 333, 1414–1415.
- OECD (Organisation for Economic Co-operation and Development), 1993. Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles. OECD, Paris, p. 79.
- Renwick, A.G., 1991. Safety factors and establishment of acceptable daily intakes. *Food Addit. Contam.* 8 (2), 135–150.
- Renwick, A.G., 1993. Data-derived safety factors for the evaluation of food additives and environmental contaminants. *Food Addit. Contam.* 10 (3), 275–305.
- Rosling, H., 1994. Measuring effects in humans of dietary cyanide exposure from cassava. In: Bokanga, M., Essers, A.J.A., Poulter, N., Rosling, H., Tewe, O. (Eds.) *International Workshop on Cassava Safety*. Ibadan, Nigeria, March 1–4, 1994. *Acta Horticulturae* 375, 271–284.
- Rothman, K.J., Moore, I.L., Singer, M.R., Nguyen, U., U-SDT, Mannino S., Milunsky, A., 1995. Teratogenicity of high vitamin A intake. *N. Engl. J. Med.*, 333, 1369–1373.
- Scambia, G., Ranelletti, F.O., Benedetti-Panici, P., Piantelli, M., Bonanno, G., De Vincenzo, R., Ferrandina, G., Pierelli, L., Capelli, A., Mancuso, S., 1991. Quercetin inhibits the growth of multidrug-resistant estrogen receptor negative MCF-7 human breast cancer cell line expressing type II estrogen binding sites. *Cancer Chemother. Pharmacol.* 28, 255–258.
- Schultz, V., 1984. Clinical pharmacokinetics of nitroprusside, cyanide, thiosulfate and thiocyanate. *Clin. Pharmacokinet.* 9, 239–251.
- Setchell, K.D.R., Zimmer-Nechemias, L., Cai, J., Heubi, J.E., 1997. Exposure of infants to phyto-estrogens from soy-based infant formula. *Lancet* 350 (July 5), 23–27.
- Speijers, G.J.A., 1995. Toxicological data needed for safety evaluation and regulation on inherent plant toxins. *Natural Toxins* 3, 222–226.
- Steinmetz, K.A., Potter, J.D., 1991a. Vegetables, fruit and cancer. I. Epidemiology. *Cancer Causes Control* 2, 325–357.
- Steinmetz, K.A., Potter, J.D., 1991b. Vegetables, fruit and cancer. II. Mechanisms. *Cancer Causes Control* 2, 427–441.
- Størmer, F.C., Reistad, R., Alexander, J., 1993. Adverse Health effects of glycyrrhizic acid in licorice—a risk assessment. Nordiske Seminar—og Arbejdsrapporter, 1993-526, Nordic Council of Ministers, Copenhagen, Denmark.
- Tiedink, H.G.M., 1991. Occurrence of indole compounds in some vegetables: toxicological implications of nitrosation with emphasis on mutagenicity. PhD Thesis, Agricultural University, Wageningen, The Netherlands. p. 157.
- Tylleskär, T., 1994. The causation of konzo—Studies on a paralytic disease in Africa. Doctoral dissertation, Uppsala University, Sweden.
- Vainio, H., Sorsa, M., McMichael, A.J., 1990. Complex mixtures and cancer risk. IARC Scientific Publications No. 104. International Agency for Research on Cancer. Lyon, France.
- Van Gelder, W.M.J., 1989. Steroidal glycoalkaloid in solanum species: consequences for potato breeding and food safety. PhD Thesis LU Wageningen, The Netherlands.
- Van Logten, M.J., De Vries, T., Van der Heijden, C.A., Van Leeuwen, F.X.R., Garbis Berkvens, J.M., Strik, J.J.T.W.A., 1983. Long-term wholesomeness study of autoclaved or irradiated pork in rats. National Institute of Public Health (RIVM), Report nr. 617401001, Bilthoven, The Netherlands.

- Verhoeven, D.T.H., Goldbohm, R.A., Van Poppel, G., Verhagen, H., Van den Brandt, P.A., 1996. Epidemiological studies on Brassica vegetables and cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 5, 733–748.
- Wattenberg, L.W., 1979. Naturally occurring inhibitors of chemical carcinogenesis. In: Miller, E.C., et al. (Eds.), *Naturally Occurring Carcinogens-mutagens and Modulators of Carcinogenesis*. Japan Scientific Society Press, Tokyo/University Park Press, Baltimore, pp. 315–328.
- Whitten, P.L., Lewis, C., Naftolin, F., 1993. A phytoestrogen diet induces the premature anovulatory syndrome in lactationally exposed female rats. *Biol. Reprod.* 49 (5), 1117–1121.
- Whitten, P.L., Russell E, E., Naftolin, F., 1994. Influence of phytoestrogen diets on estradiol action in the rat uterus. *Steroids* 59 (7), 443–449.
- Whitten, P.L., Lewis, C., Russell, E., Naftolin, F., 1995. Phytoestrogen influences on the development of behavior and gonadotropin function. *Proc. Soc. Exp. Biol. Med.* 208 (1), 82–86.
- Zhu, B.T., Liehr, J.G., 1994. Quercetin increases the severity of estradiol-induced tumorigenesis in hamster kidney. *Toxicol. Appl. Pharmacol.* 125 (1), 149–158.